REMARKS

Claims 62-64, 68, 69, 92, 100, 104, 120, 121, 128, and 129 have been canceled without prejudice or disclaimer. New claims 131-151 have been added. Claims 1, 52, 53, and 130 have been amended. Claims 1, 24, 32, 36, 52, 53, 60, 61, and 130-151 are pending in the present application.

It is respectfully submitted that the present amendment presents no new issues or new matter and places this case in condition for allowance.

I. Objections - Abstract

The Office Action noted that the Abstract lists the amino acid substitutions merely by residue number, except for residue 193, which is listed as the specific substitution S193A and whether such inconsistency was Applicant's intention.

Applicant has amended the term "S193A" to recite "193".

II. Objections - Claims

The Office Action objected to claims 36, 61, and 130 for having improper antecedent usage as follows. Claim 36: "the insertion G224GT" has no antecedent basis. Claim 61: the phrases "the form of a precursor" and "the prepro region" have no antecedent basis. Claim 130: "a variant of claim 1" should be "the variant of claim 1".

Applicant has corrected the improper antecedent usage in the amended claims.

II. The Rejection of Claims 1, 24, 36, 52, 53, 61, and 130 under 35 U.S.C. § 112, Second Paragraph

Claims 1, 24, 36, 52, 53, 61, and 130 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite on several grounds.

Ground 1: The Office Action states that the phrase "corresponding to positions ... of SEQ ID NO: 2" renders claim 1 indefinite because it is unclear whether the scope of claim 1 encompasses only variants of the protein set forth by SEQ ID NO: 2 or variants of any protein having residues corresponding to those in SEQ ID NO: 2.

The phrase "corresponding to positions... of SEQ ID NO: 2" encompasses variants of any protein having residues corresponding to those in SEQ ID NO: 2. The phrase "positions corresponding to positions" of amino acids 25 to 248 of SEQ ID NO: 2, or various wording

thereof, is defined in the specification on page 5, lines 28-30, as analogous positions of the microbial trypsin that correspond to amino acids 25 to 248 of SEQ ID NO: 2.

Ground 2: The Office Action stated that the phrase "hybridizes under at least low stringency conditions" of claim 1 is indefinite because the term is unclear absent a statement of the conditions under which the hybridization reaction is performed since the hybridization conditions described on page 10 lines 22-30 are only exemplary and do not define the conditions recited in claim 1.

Claim 1 has been amended to recite the following stringency conditions: "low stringency conditions are defined as prehybridization and hybridization at 42°C in 5X SSPE, 0.3% SDS, 200 µg/ml sheared and denatured salmon sperm DNA, and 25% formamide followed by washing three times each for 15 minutes using 2X SSC, 0.2% SDS at 50°C". Conditions for other stringency conditions have also been defined in several newly added claims.

Ground 3: The Office Action states that claims 32 and 36 are rendered indefinite by "V192*" "K197*", and "A226*" because a person of ordinary skill in the art would not know the metes and bound of the recited invention. This rejection is respectfully traversed.

"VI92*" "KI97*", and "A226*" are defined on page 7, lines 17-20, of the specification, under the Deletion Section, which states: "For an amino acid deletion, the following nomenclature is used: [Original amino acid; Position*]. Accordingly, the deletion of glycine at position 195 is designated as "Gly195*" or "G195*."

Ground 4: The Office Action states that claims 52 and 53 are rendered indefinite by the phrase "preferably" because it is unclear whether the limitations preceding the phrase are part of the claimed invention. Claims 52 has been amended to recite: "The variant of claim 1, wherein the total number of substitutions is at least 1 and at the most 11." Claim 53 has been amended to recite: "The variant of claim 1, wherein the total number of deletions is at least 1 and at the most 3."

For the foregoing reasons, Applicants submit that the claims overcome the rejections under 35 U.S.C. § 112. Applicants respectfully request reconsideration and withdrawal of the rejection.

III. The Rejection of Claims 1, 24, 36, 52, 53, 61, and 130 under 35 U.S.C. § 112, First Paragraph

Claims 1, 24, 36, 52, 53, 61, and 130 stand rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement. The Office Action stated:

> Claims 1, 24, 36, 52, 53, 61, and 130 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for the variant of SEQ ID NO: 2, wherein said variant has the substitutions V144T, S193A, DI98S, Q201M, A218I, N223S, or R227S, P228T, N229S, Y230T, and S231P, deletion of residues 192, 197, and 226, and insertion of a threonine between residues 224 and 225 and has chymotrypsin-like activity (Fig 6), does not reasonably provide enablement for any variant of any microbial trypsin, wherein the variant has chymotrypsin-like activity and has a substitution, relative to SEQ ID NO: 2, at one or more of residues corresponding to 144, 193, 198, 201, 218, 223, or 227-231, a deletion at one or more of residues corresponding to 192, 197, or 226, and an insertion between residues 224 and 225 and has either (a) 70% homology to residues 25-248 of SEQ ID NO: 2 or (b) hybridizes to residues 202-801 of SEQ ID NO: 1.

This rejection is respectfully traversed.

Applicants submit that the specification complies with the enablement requirement.

Section 112 of the U.S. Patent Code requires that the specification be "enabling" to a person skilled in the art to which the invention pertains. "A specification disclosure which contains a teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as in compliance with the enabling requirement of the first paragraph of section 112 unless there is reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support." *In re Marzocchi*, 169 USPQ at 369.

It is also well settled that an assertion by the Patent Office that the enabling disclosure is not commensurate in scope with the protection sought must be supported by evidence or reasoning substantiating the doubts so expressed. *In re Dinh-Nguyen*, 181 U.S.P.Q. 46 (C.C.P.A. 1974). See also *U.S. v. Telectronics*, 8 U.S.P.Q.2d 1217 (Fed. Cir. 1988); *In re Bowen*, 181 U.S.P.Q. 48 (C.C.P.A. 1974); *Ex parte Hitzeman*, 9 U.S.P.Q.2d 1821 (BPAI 1988). In the absence of any evidence or apparent reason why compounds do not possess the disclosed utility, the allegation of utility in the specification must be accepted as correct. *In re Kamal*, 158 U.S.P.Q. 320 (C.C.P.A. 1968). See also *In re Stark*, 172 U.S.P.Q. 402, 406 n. 4 (C.C.P.A. 1972) (the burden is upon the Patent Office to set forth reasonable grounds in support of its contention that a claim reads on inoperable subject matter).

Enablement requires that the specification teach those in the art to make and use the invention without undue experimentation. *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988). As stated in *Wands*, [w]hether undue experimentation is needed is not a single, simple factual determination, but rather is a conclusion reached by weighing many factual considerations."

See id. at 1404. The *Wands* factors which may be relevant for determining whether a disclosure would require undue experimentation include (1) the quantity of experimentation necessary; (2) the amount of direction or guidance presented; (3) the presence or absence of working examples; (4) the nature of the invention; (5) the state of the prior art; (6) the relative skill of those in the art; (7) the predictability or unpredictability of the art; and (8) the breadth of the claims. *Id.*

Applicants disagree with the Office's contention that the specification does not reasonably provide enablement for any variant of any microbial trypsin, wherein the variant has chymotrypsin-like activity and has a substitution at one or more positions corresponding to positions 144, 193, 198, 201, 218, 223, 227, 228, 229, 230, and 231 of amino acids 25 to 248 of SEQ ID NO: 2, a deletion at one or more positions corresponding to positions 192, 197, and 226 of amino acids 25 to 248 of SEQ ID NO: 2; and an insertion between positions corresponding to positions 224 and 225 of amino acids 25 to 248 of SEQ ID NO: 2, and has either (a) 70% identity to residues 25-248 of SEQ ID NO: 2 or (b) hybridizes to residues 202-801 of SEQ ID NO: 1.

The Office Action asserts that the "scope of each of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of polypeptides broadly encompassed by the claim" because "the amino acid sequence of a protein determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired chymotrypsin-like activity requires a knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant to modification), and detailed knowledge of the ways in which the protein's structure relates to its function." The Office Action also asserts that the "specification does not support the broad scope of Claims 1, 24, 36, 52, 53, 61, and 130, which encompass the microbial trypsin variants described above, because the specification does not establish: (A) regions of the protein structure which may be modified without effecting the chymotrypsin activity; (B) the general tolerance of the chymotrypsin activity to modification and extent of such tolerance; (C) a rational and predictable scheme for modifying any residues with an expectation of obtaining the desired biological function; and (D) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful." Applicants disagree with these assertions.

Applicants disclose methods and examples for the construction of variants with

chymotrypsin-like activity from a microbial trypsin by identifying the positions of the amino acids in the microbial trypsin that correspond to the amino acids of a chymotrypsin responsible for catalytic activity and substituting, deleting, and/or inserting amino acids in the microbial trypsin to correspond to the same and/or similar amino acids of the chymotrypsin by site-directed mutagenesis or any other suitable method known in the art. The amino acids of a chymotrypsin responsible for catalytic activity are well known in the art and include not only the amino acids involved in enzyme catalysis, but also the amino acids of the binding site and surface loops of the binding pocket.

Identification of such amino acids in the microbial trypsin is accomplished by aligning the amino acid sequence of the microbial trypsin with the amino acid sequences of one or more chymotrypsins and/or by comparing the secondary or 3D structures of the microbial trypsin and one or more chymotrypsins. Applicants provide details on page 6, line 2, to page 7, line 9, of the specification of how to indicate the position of an amino acid residue in a microbial trypsin in regions of structural homology using the numbering system originating from the amino acid sequence of the microbial trypsin disclosed in SEQ ID NO: 2, aligned with the amino acid sequence of another microbial trypsin. The amino acid sequence of a trypsin-like protease, *e.g.*, *Fusarium oxysporum* trypsin-like protease, can be aligned to the amino acid sequence of bovine chymotrypsin A (SWISSPROT P00766) to ascertain the amino acids corresponding to the catalytic site amino acids of the bovine chymotrypsin.

Applicants describe the catalytic region and the amino acid positions of a microbial trypsin protein required to be mutated to produce a microbial trypsin mutant having chymotrypsin-like activity using amino acids 25 to 248 of SEQ ID NO: 2 as a reference protein. These mutations include (1) a substitution at one or more positions corresponding to positions 144, 193, 198, 201, 218, 223, 227, 228, 229, 230, and 231 of amino acids 25 to 248 of SEQ ID NO: 2, (2) a deletion at one or more positions corresponding to positions 192, 197, and 226 of amino acids 25 to 248 of SEQ ID NO: 2; and (3) an insertion between positions corresponding to positions 224 and 225 of amino acids 25 to 248 of SEQ ID NO: 2. See the "Construction of Microbial Trypsin Variants with Chymotrypsin-like Activity" section beginning on page 13, and the "Variants" section beginning on page 18 of the specification. Moreover, Example 1 of the specification provides a working example of how to engineer a *Fusarium oxysporum* trypsinogen-like gene so as to encode variants having chymotrypsin-like activity.

Applicants submit that the specification provides a rational and predictable scheme for modifying the designated amino acid residues with an expectation of obtaining microbial trypsin

variants having chymotrypsin-like activity. The specification describes methods for obtaining microbial trypsin-like or trypsinogen-like proteins on page 9, line 21, to page 13, line 14; methods for identifying the positions of the amino acids in the microbial trypsin that correspond to the amino acids of a chymotrypsin responsible for catalytic activity on page 6, line 1, to page 7, line 9; methods for constructing microbial trypsin variants with chymotrypsin-like activity using various techniques known in the art such as site-directed mutagenesis on page 13, line 16, to page 18, line 12, and Example 1; methods for expressing the microbial trypsin variants having chymotrypsin-like activity on page 29, line 15, to page 40, line 11, and Examples 2 and 3; methods for assaying for trypsin and chymotrypsin activity on page 4, line 28, to page 5, line 1, and page 5, line 13-20; and methods for purifying and characterizing the microbial trypsin variants having chymotrypsin-like activity in Examples 4-7.

The Office action suggests that the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful. Applicants disagree with this assertion. The specification is very clear about what amino acid positions to mutate as described above. In fact, Applicants in Example 1 describe how to mutate these positions and show in later Examples that a microbial trypsin variant has chymotrypsin-like activity.

The Examiner's conclusions fail to give appropriate consideration to the high level of skill in the art. As of the time of the invention, it was routine for persons of ordinary skill in the art to prepare and screen for variants of SEQ ID NO:2 which encode a protein that possess at least 70% identity to SEQ ID NO:2 and has chymotrypsin-like activity. Indeed, as of September 2002, persons of ordinary skill in the art were able to routinely produce and screen in a very short period of time hundreds of thousands of mutants of a known sequence through mutagenesis and other techniques.

The information in the public domain also provides detailed guidance to assist an artisan when preparing microbial trypsin variants having chymotrypsin-like activity including detailed guidance of both conserved sequences, sequence which are important for function, and sequences which can be altered without disrupting chymotrypsin activity, as well as detailed information of the ways in which the protein's structure relates to its function. In particular, Hedstrom *et al.*, 1992, *Science* 255: 1240-1253, disclose the protein engineering of a mammalian trypsin gene to code for a polypeptide with a functional chymotrypsin substrate profile by site-directed mutagenesis of the S1 binding site and surface loops of the binding pocket of trypsin with analogous residues of chymotrypsin.

This teaching, coupled with Applicants' specification and examples and the ability to test

for functional mutants with the assays provided in the specification, provide sufficient guidance to enable an artisan to make combinations of the mutations disclosed for SEQ ID NO: 2 in other microbial trypsins. Such work is certainly not undue, as the production of variants using this technology was routine in the art as of the filing of the application. Thus, although Applicants are not suggesting that one skilled in the art go out and make all of the possible combinations of the alterations disclosed, and such information is not necessary to establish enablement of the claimed invention, the technology available to the artisan at the time of the invention nevertheless enabled the artisan to carry such tasks out.

Accordingly, the specification enables the claimed invention because the specification contains a teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and defining the subject matter.

For the foregoing reasons, Applicants submit that the new claims overcome the rejections under 35 U.S.C. § 112. Applicants respectfully request reconsideration and withdrawal of the rejection.

IV. The Rejection of Claim 60 under 35 U.S.C. § 112, First Paragraph

Claim 60 stands rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement. The Office Action stated:

> The invention of Claim 60 appears to employ a novel vector. Since the vector is essential to the claimed invention, it must be obtainable by a repeatable method set forth in the specification or otherwise be readily available to the public. The claimed plasmid's sequence is not fully disclosed, nor have all the sequences required for its construction been shown to be publidy known and freely available. The enablement requirements of35 U.S.C. § 112 may be satisfied by a deposit of the plasmid. The specification does not disclose a repeatable process to obtain the vector and it is not apparent if the DNA sequences are readily available to the public. Accordingly, it is deemed that a deposit of these plasmids should have been made in accordance with 37 CFR 1.801-1.809. It is noted that applicants have deposited the organisms but there is no indication in the specification as to public availability. If the deposit is/was made under the terms of the Budapest Treaty, then an affidavit or declaration by applicants, or a statement by an attorney of record over his or her signature and registration number, stating that the specific strain has been deposited under the Budapest Treaty and that the strain will be available to the public under the conditions specified in 37 CPR 1.808, would satisfy the deposit requirement made herein.

Applicants enclose a Statement under 37 C.F.R. § 1.808 that *E. coli* pEJG66.1XLGOLD was deposited under the Budapest Treaty and all restrictions will be removed upon the granting of the U.S. patent.

For the foregoing reasons Applicants submit that the claims overcome the rejections under 35 U.S.C. § 112. Applicants respectfully request reconsideration and withdrawal of the rejection.

V. The Rejection of Claims 1, 24, 36, 52, 53, 61, and 130 under 35 U.S.C. § 112, First Paragraph

Claims 1, 24, 36, 52, 53, 61, and 130 stand rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. The Office Action stated:

> These claims are directed to a genus of polypeptide having chymotrypsin-like activity, wherein the polypeptide has, relative to SEQ ID NO: 2, a substitution at one or more of residues corresponding to 144, 193, 198, 201, 218, 223, or 227-231, a deletion at one or more of residues corresponding to 192, 197, or 226, an insertion between residues 224 and 225, and has either (a) 70% homology to residues 25-248 of SEQ ID NO: 2 or (b) hybridizes to residues 202-801 of SEQ ill NO: 1. The specification teaches the structure of only a single representative species of such polypeptides. Moreover, the specification fails to describe any other representative species by any identifying characteristics or properties other than the functionality of having chymotrypsin-like activity. Given this lack of description of representative species encompassed by the genus of the claim, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

This rejection is respectfully traversed.

The written description requirement of 35 U.S.C. § 112, first paragraph, is fulfilled when the patent specification describes the claimed invention in sufficient detail such that the claim limitations are described so that one of skill in the art would recognize that the applicants had invented the subject matter. *See Vas-Cath, Inc. v. Mahurkar*, 19 USPQ2d 1111, 1116 (Fed. Cir. 1991), *In re Herschler*, 591. F.2d 693, 700 (CCPA 1979). The written description as filed is presumed to be adequate, unless or until sufficient evidence or reasoning to the contrary has been presented by the examiner to rebut the presumption. *See In re Marzocchi*, 169 USPQ 367 (CCPA 1971). It is well settled that "[t]he test for determining compliance with the written description requirement is whether the disclosure of the application as originally filed reasonably conveys to the artisan that the inventor had possession at that time of the later claimed subject matter ..." *In re Kaslow*, 217 USPQ 1089, 1096 (Fed. Cir. 1983).

The written description requirement can be met by showing that an invention is complete by disclosure of sufficiently detailed, relevant identifying characteristics, *i.e.*, complete or partial

structure, other physical and/or chemical properties, functional characteristics when coupled with know or disclosed correlation between function and structure, or some combination of such characteristics. See, e.g., *University of California v. Eli Lilly and Co.*, 43 U.S.P.Q.2d 1398, 1404 (Fed. Cir. 1997); *Enzo Biochem v. Gen-Probe Inc.*, 63 U.S.P.Q.2d 1609, 1613 (Fed. Cir. 2002).

The Examiner's written description rejection primarily focuses on the assertion that the specification fails to describe any other representative species by any identifying characteristics or properties other than the functionality of having chymotrypsin-like activity. Applicants disagree with this assertion.

It is well-established in the art that the definition of a genus of polypeptides having an enzyme activity of interest is accomplished by using structural features that show the relatedness of the genes and their encoded products which are members of the genus. For decades the scientific community has relied on the structural features of (1) percent identity of the amino acid sequences encoded by the genes and/or (2) nucleic acid hybridizations under defined stringent conditions as structural features to reasonably predict the function of polypeptides encoded by genes, and to place the genes and encoded polypeptides into an existing genus. In particular, polypeptides having a high degree of sequence similarity to another polypeptide are expected to have a very similar function. Nucleic acids having a high degree of sequence similarity to another nucleic acid are likewise expected to encode a polypeptide having a very similar function. Nucleic acids which hybridize to another nucleic acid under certain stringent conditions are also expected to encode a polypeptide having a very similar function. The U.S. Patent Office and patent authorities throughout the world have also accepted these structural features to define a genus of genes or proteins, as evidenced by the numerous issued patents containing these structural features.

The instant claims recite the structural features that (1) the mutations include (a) a substitution at one or more positions corresponding to positions 144, 193, 198, 201, 218, 223, 227, 228, 229, 230, and 231 of amino acids 25 to 248 of SEQ ID NO: 2, (b) a deletion at one or more positions corresponding to positions 192, 197, and 226 of amino acids 25 to 248 of SEQ ID NO: 2; and (c) an insertion between positions corresponding to positions 224 and 225 of amino acids 25 to 248 of SEQ ID NO: 2; (2) that the microbial trypsin is (a) a polypeptide comprising an amino acid sequence which has at least 70% identity to amino acids 25 to 248 of SEQ ID NO: 2; or (b) a polypeptide encoded by a nucleotide sequence which hybridizes under at least low stringency conditions with nucleotides 202 to 801 of SEQ ID NO: 1 or its complementary strand, wherein low stringency conditions are defined as prehybridization and hybridization at 42°C in

5X SSPE, 0.3% SDS, 200 µg/ml sheared and denatured salmon sperm DNA, and 25% formamide followed by washing three times each for 15 minutes using 2X SSC, 0.2% SDS at 50°C; and (3) the variant has chymotrypsin-like activity.

The structural feature of 70% identity to amino acids 25 to 248 of SEQ ID NO: 2 as a reference polypeptide inherently defines the function of the encoded products and provides a reasonable prediction of relatedness and the identification of members of the genus. In particular, it is accepted in the art that polypeptides having at least 70% identity to a reference polypeptide are reasonably expected to have a very similar function as the reference polypeptide. The structural feature that the variants with chymotrypsin-like activity have a substitution at one or more positions corresponding to positions 144, 193, 198, 201, 218, 223, 227, 228, 229, 230, and 231 of amino acids 25 to 248 of SEQ ID NO: 2, a deletion at one or more positions corresponding to positions 192, 197, and 226 of amino acids 25 to 248 of SEQ ID NO: 2; and an insertion between positions corresponding to positions 224 and 225 of amino acids 25 to 248 of SEQ ID NO: 2 also defines the function of the variants and provides a reasonable prediction of relatedness and the identification of members of the genus. The above mutated residues correspond to amino acids of a chymotrypsin responsible for catalytic activity and are well known in the art.

In addition to structural features common to the members of the genus, the possession of the recited genus is also shown by the description of a representative number of species falling within the scope of the genus. Example 1 discloses the construction of various variants. The final variant constructed in Example 1 had the substitutions V144T, S193A, D198S, Q201M, A218I, N223S, R227S, P228T, N229S, Y230T, and S231P, deletions of V192, K197, and A226, and a Thr was inserted between G224 and C225.

Moreover, the claimed invention need not be described in *haec verba* to satisfy the description requirement as the relevant analysis is whether the claim limitations were described so that one of skill in the art would recognize that the applicants had invented the claimed subject matter. As discussed above, Applicants have met this showing by a recitation of structural features which are common to the members of the genus, and which distinguishes the genus from other subject matter, and/or by a recitation of a representative number of nucleic acid falling within the scope of the genus.

In sum, Applicants' specification provides a precise definition by structure of the genus of materials sufficient to distinguish it from other materials and a description of the genus by recitation of a representative number of species falling within the scope of the genus. See, e.g.,

University of California v. Eli Lilly and Co., 43 U.S.P.Q.2d 1398, 1404 (Fed. Cir. 1997); *Enzo Biochem v. Gen-Probe Inc.*, 63 U.S.P.Q.2d 1609, 1613 (Fed. Cir. 2002. Accordingly, based on the disclosure provided in the specification, the written description requirement is satisfied as the description provides sufficient information to establish that Applicants were in possession of the claimed microbial trypsin variants having chymotrypsin-like activity.

For the foregoing reasons, Applicants submit that the claims overcome the rejections under 35 U.S.C. § 112. Applicants respectfully request reconsideration and withdrawal of the rejection.

VI. Conclusion

In view of the above, it is respectfully submitted that all claims are in condition for allowance. Early action to that end is respectfully requested. The Examiner is hereby invited to contact the undersigned by telephone if there are any questions concerning this amendment or application.

Respectfully submitted,

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